

Amendments to the Claims

1. (Currently Amended) A method of producing male or female sterile plants comprising the steps of:
 - a) transforming plant material with a polynucleotide which encodes at least one enzymewhich reacts with a non-phytotoxic substance to produce a phytotoxic one, wherein the enzyme is expressed preferentially in either male or female reproductive structures and characterized in that the enzyme is a mutant D-amino acid oxidase, derived from *Rhodotorula gracilis*, said oxidase comprising a lysine at position 58 rather than a phenylalanine in the wild type sequence, and wherein the non-phytotoxic substance is characterized as a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid;[[, and]]
 - b) regenerating the thus transformed material into a plant;[[,]]
 - c) wherein applying the said non-phytotoxic substance is applied to the plant up to the time of male or female gamete formation and/or maturation, so that the non-phytotoxic substance provides for the production of a phytotoxic one which selectively prevents the formation of or otherwise renders the said gametes non-functional[[,]] ; and
 - d) selecting for the plant comprising either male or female reproductive structures.

~~wherein the enzyme is expressed preferentially in either male or female reproductive structures; and the non-phytotoxic substance is a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid, characterised in that the enzyme is a mutant D-amino acid oxidase, obtainable from *Rhodotorula gracilis*, which oxidase comprises a lysine at position 58 rather than a phenylalanine in the wild type sequence.~~
2. (Original) A method according to claim 1, wherein the said non-phytotoxic substance is applied in mixture along with at least one further substance which is selected from the group consisting of safeners, gametocides, glutathione-S-transferase inducers, cytochrome P450 inducers, herbicides, fertilizers, nematocides, synergists, insecticides, fungicides, hormones, plant-growth regulators and cytochrome P450 inhibitors.
3. (Previously Amended) A method according to claim 1, wherein the non-phytotoxic substance is applied foliarly and is a phloem mobile and metabolically stable oxidisable substrate of the enzyme, wherein the enzyme provides the phytotoxic product, as a direct or indirect one from the non-phytotoxic substance.
4. (Currently Amended) A method according to the claim 3, wherein the phytotoxic product is an indirect one produced in the form of peroxide ~~and/or a super oxide anion.~~

5. (Previously Amended) A method according to claim 3, wherein the non-phytotoxic substance is D-aspartate or D-glutamate and the enzyme oxidises the said amino acid to a 2-keto acid with concomitant reduction of oxygen to a peroxide anion.
6. (Currently Amended) A method according to claim 1 wherein the enzyme comprises a substitution[[s]] at position[[s]] 213,~~223 and/or 238~~ when compared to the wild type sequence.
7. (Currently Amended) A method according to claim 6, wherein the ~~oxidase enzyme~~ enzyme has at position 213 an amino acid selected from the group consisting of His:, Ser, Thr, Cys, Gln, Gly, Asn and Ala,~~and/or at position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Asn, Gln, Gly and Ala, and/or at position 223 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Ala, Gly, Gln and/or Asn.~~
8. (Currently Amended) A method according to claim 7 wherein the amino acid at position 213 is selected from the group comprising Ser and [[or]] Thr.
9. (Previously Amended) A method according to claim 3, wherein the enzyme is targeted to other than the peroxisome.
10. (Currently Amended) A method according to claim 1, wherein the non-phytotoxic substance is one or more substances either selected from the group comprising the D₋ enantiomer of phosphinothricin [[or]] and a D₋ enantiomer of bialaphos.
11. (Previously Amended) A method according to claim 1, wherein the non-phytotoxic substance is comprised within a mixture, which contains a phytotoxic substance and wherein the enzyme oxidises an amino acid to a 2-keto acid with concomitant reduction of oxygen to a peroxide anion.
12. (Currently Amended) A method according to claim 11 wherein the enzyme is a mutant D-amino acid oxidase ~~obtainable~~ derived from *Rhodotorula gracilis* which oxidase comprises substitutions ~~at positions in at least one position selected from the group comprising 213, and/or 238, and/or or 223~~ when compared to the wild type sequence,~~or is a D-aspartate oxidase.~~
13. (Currently Amended) A method according to claim 12, wherein the oxidase ~~obtainable~~ derived from *Rhodotorula gracilis* has at position 213 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, ~~and/or at position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and at position 223 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala.~~
14. (Currently Amended) A method according to claim 13 where the amino acid at position 213 is selected from the group comprising Ser and [[or]] Thr.
15. (Currently Amended) A method according to claim 10, wherein the mixture comprises both D₋ and L₋ phosphinothricin and the plant material expresses a PAT gene substantially ~~only~~ in

green tissues and/or in floral tissue which produce gametes being other than those that are rendered non-functional.

16. (Currently Amended) A mutant D-amino acid oxidase ~~obtainable~~ derived from *Rhodotorula gracilis*, capable of oxidising phosphinothricin, which comprises a lysine at position 58 rather than a phenylalanine in the wild type sequence.

17. (Currently Amended) An oxidase according to claim ~~14~~16, further comprising amino acid substitutions in at least one position selected from the group consisting of 213, 223, and 238.

18. (Currently Amended) A method of producing male or female sterile plants comprising the steps of:

- a) transforming plant material with a polynucleotide which encodes at least one enzymewhich reacts with a non-phytotoxic substance to produce a phytotoxic one, wherein the enzyme is expressed preferentially in either male or female reproductive structures and characterized in that the enzyme is a mutant D-amino acid oxidase, derived from *Rhodotorula gracilis*, said oxidase comprising a lysine at position 58 rather than a phenylalanine in the wild type sequence and a serine at position 213 rather than a methionine in the wild type sequence, and wherein the non-phytotoxic substance is characterized as a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid;[, and]]
- b) regenerating the thus transformed material into a plant;[,]]
- c) wherein applying the said non-phytotoxic substance is applied to the plant up to the time of male or female gamete formation and/or maturation, so that the non-phytotoxic substance provides for the production of a phytotoxic one which selectively prevents the formation of or otherwise renders the said gametes non-functional;[,]]; and
- d) selecting for the plant comprising either male or female reproductive structures.

~~wherein the enzyme is expressed preferentially in either male or female reproductive structures; and the non-phytotoxic substance is a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid, characterised in that the enzyme is a mutant D-amino acid oxidase, obtainable from *Rhodotorula gracilis*, which oxidase comprises a lysine at position 58 rather than a phenylalanine in the wild type sequence; and a serine at position 213 rather than a methionine in the wild type sequence~~

19. (New) The method according to claim 3, wherein the phytotoxic product is an indirect one produced in the form of a super oxide anion.

20. (New) The method according to claim 1 wherein the enzyme comprises a substitution at position 223 when compared to the wild type sequence.

21. (New) The method according to claim 1 wherein the enzyme comprises a substitution at position 238 when compared to the wild type sequence.
22. (New) The method according to claim 7, wherein the enzyme has at position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Asn, Gln, Gly and Ala.
23. (New) The method according to claim 7, wherein the enzyme has at position 223 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Ala, Gly, Gln and Asn.
24. (New) The method according to claim 16 wherein the enzyme is a mutant D-amino acid oxidase obtainable derived from *Rhodotorula gracilis* which oxidase is a D-aspartate oxidase.
25. (New) The method according to claim 12, wherein the oxidase obtainable derived from *Rhodotorula* has position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and at position 223 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala.
26. (New) The method according to claim 15, wherein the mixture comprises both D- and L-phosphinothricin and the plant material expresses a PAT gene substantially in floral tissue which produce gametes being other than those that are rendered non-functional.